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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/18, C12Q 1/68, G01N 33/53

A1

(11) International Publication Number: **WO 98/19696**

(43) International Publication Date: 14 May 1998 (14.05.98)

(21) International Application Number: PCT/US97/19891

(22) International Filing Date: 3 November 1997 (03.11.97)

(30) Priority Data:
60/030,342 5 November 1996 (05.11.96) US

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(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HEPATOCYTE GROWTH FACTOR ANTAGONISTS

(57) Abstract

A method of treating a human having chronic renal disease is provided comprising administering a hepatocyte growth factor antagonist to the human.

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HEPATOCYTE GROWTH FACTOR ANTAGONISTS

Field of the Invention

The field of the invention is treatment of chronic renal disease.

Background of the Invention

Hepatocyte growth factor (HGF) is a heterodimeric molecule derived from a preproprecursor protein of 728 amino acids, which is proteolytically processed by a specific protease to form mature HGF (Miyazawa et al., 1993, J. Biol. Chem. 268:10024-10028).

HGF binds to cells through the tyrosine kinase receptor c-met. Binding of HGF to c-met has the following effects: Growth of renal epithelial cells is stimulated; the motility of cells is enhanced; and, renal tubule formation is induced (Santos et al., 1993, Dev. Biol. 159:535-548; Cantley et al., 1994, Am. J. Physiol. 267:F271-F280). Thus, HGF may be characterized as a mitogen, a motogen and a morphogen, respectively, and is believed to play a role in renal development.

In addition, HGF is involved in renal remodeling following injury as reflected by increased levels of HGF and its receptor c-met in the kidney following nephrectomy or ischemia (Joannidis et al., 1994, Am. J. Physiol. 267:F231-F236). Further, HGF has been observed to effect an improvement in renal function following mercuric chloride assault or ischemia (Kawaida et al., 1994, Proc. Natl. Acad. Sci. USA 91:4357-4361; Miller et al., 1994, Am. J. Physiol. 266:F129-F134). Glomerular hypertrophy occurs during renal remodeling in many chronic renal diseases. This event is associated with glomerular basement membrane expansion, proliferation of mesangial and epithelial cells, and the accumulation of

collagen and fibronectin. The role of HGF in glomerular extracellular matrix expansion is not known.

Chronic renal failure is the progressive loss of functional renal mass, accompanied by compensatory growth and remodeling. The molecular and cellular events that take place during chronic renal failure include release of growth factors, proliferation of glomerular mesangial cells and expansion of extracellular matrix (Klahr et al., 1988, New Engl. J. Med. 318:1657-1666; Striker et al., 1989, In: Klahr, (Ed.) Seminars in Nephrology, pp. 318, Philadelphia, W.B. Saunders); Ebihara et al., 1993, J. Am. Soc. Nephrol. 3:1387-1397). The components of the extracellular matrix which change during chronic renal failure include collagen, fibronectin, and laminin (Ebihara et al., *supra*; Tarsio et al., 1988, Diabetes 37:532-539; Yoshioka et al., 1989, Kidney Int. 35:1203-1211). In the renal ablation model, fibronectin and collagen a1 immunocytochemical staining is elevated in areas of increased cellularity of glomeruli at 2 and 6 weeks after nephrectomy of 5/6 of the renal mass, when chronic renal failure is established (Foelge et al., 1992, Lab. Invest. 66:485-497).

Several protein factors have been implicated in stimulating mitogenesis of mesangial cells or extracellular matrix production during chronic renal failure. These include thrombin (Xu et al., 1995, Am. J. Pathol. 146:101-110; Sraer et al., 1993, Ren. Fail. 15:343-348; Albrightson et al., 1992, J. Pharmacol. Exp. Ther. 263:404-412), epidermal growth factor (Byyny et al., 1972, Endocrinology 90:1261-1266; Killion et al., 1993, J. Urol. 150:1551-1556), insulin-like growth factor-1 (Stiles et al., 1985, Endocrinology 117:2397-2401; Fagin et al., 1987, Endocrinology 120:718-724), and transforming growth factor-b1 (Coimbra et al., 1991,

Am. J. Pathol. 138:223-234; Okuda et al., 1990, J. Clin. Invest. 86:453-462).

There remains a need for compositions for treatment of chronic renal disease which serve to diminish or ablate disease leading to renal failure and either death or dependence upon dialysis.

Summary of the Invention

The invention relates to a method of treating a human having chronic renal disease comprising administering a hepatocyte growth factor antagonist to the human.

Brief Description of the Drawings

Figure 1 is a series of graphs depicting extracellular acidification rates of transformed mouse mesangial cells (MMC-SV40 cells) (Panels A-C), or normal human mesangial cells (Panel D) determined by microphysiometry. Panel A: MMC-SV40 cells treated with HGF (100 ng/ml) for 10 minutes in the presence or absence of 0.1% bovine serum albumin (BSA) or 0.5% fetal bovine serum (FBS). Panel B: MMC-SV40 cells treated with HGF (100 ng/ml) for 2, 5, 10, and 15 minutes. Panel C: MMC-SV40 cells treated with 4 concentrations of HGF. Panel D: Normal human mesangial cells treated with 100 ng/ml HGF; duplicate traces are shown.

Figure 2 is a graph depicting extracellular acidification rates of MMC-SV40 cells treated with HGF (50 ng/ml). Cells were perfused with control medium or medium containing different concentrations of RO-32-0432, a protein kinase inhibitor described by Birchall et al., 1994, J. Pharmacol. Exp. Ther. 268:922-929.

Figure 3 is a graph depicting tritiated thymidine incorporation of normal human mesangial cells (HMC),

MMC-SV40 cells, or mouse mesangial cells transfected with a luciferase reporter plasmid driven by the collagen a1 (IV) promoter (MMC-COL cells) treated with different concentrations of HGF for 24 hours.

Figure 4 is a graph depicting collagen a1 (IV) promoter activity in MMC-COL cells in response to different concentrations of HGF. Data are the means ± SD of quadruplicates.

Figure 5 is a graph depicting creatinine clearance in lean and obese (diabetic) mice assessed at 21 days after either vehicle or HGF implants. HGF significantly decreased creatinine levels in obese (diabetic) mice (* < 0.05; n=5-6). LV - lean vehicle; LH - lean HGF; OV - obese vehicle; OH - obese HGF treated mice.

Detailed Descripton of the Invention

It has been discovered according to the present invention, that long term administration of HGF results in chronic renal disease. Thus, although HGF may be useful for treatment of acute renal disease, when this compound is administered to a mammal for prolonged periods of time, renal function is decreased. It has also been discovered that treatment of cells with HGF results in increased fibronectin mRNA and transcription in mesangial and epithelial cells. Thus, the present invention is based on the discovery that HGF contributes to renal disease in part by activating fibronectin and collagen synthesis, thereby causing glomerulosclerosis.

The invention relates to a method of treating chronic renal disease in a mammal comprising administering an antagonist of HGF to the mammal.

By "chronic renal disease" as used herein, is meant progressive loss of renal function as measured by glomerular filtration rate.

Antagonists of HGF include, but are not limited to, small non-peptide molecules, peptides comprising specific portions of HGF, peptidometics having anti-HGF activity, antibodies directed against HGF, nucleic acids having a sequence which is complementary to all or a portion of the nucleic acid encoding HGF and small chemical compounds which inhibit HGF-specific protease.

By "HGF antagonist activity" as used herein, is meant a compound which inhibits the normal activity of HGF, such as determined, for example, in any one or more of the assays described herein. By way of example, treatment of mouse mesangial cells with HGF results in an increase in the acidification rate of these cells. Thus, a compound having HGF antagonist activity is defined as one which inhibits an HGF-induced increase in acidification rate in mouse mesangial cells.

To identify an antagonist of HGF, a test compound is assessed for HGF antagonist activity in one or more of the assays described herein in the experimental examples section, or in any other assay for measurement of HGF function. Preferably, initially, an *in vitro* test is used to identify a compound having HGF antagonist activity. Such *in vitro* tests include, but are not limited to, tests which assess the affect of the test compound on HGF binding to cell membranes of cells which are known to respond to HGF, on HGF-induced acidification rate of mesangial cells and tests which assess the affect of the test compound on HGF-induced extracellular matrix gene expression.

To assess the effect of a test compound on HGF binding, [^{125}I]-labeled HGF is incubated with cell

membranes from A498 cells attached to scintillation beads plus or minus the test compound. If the compound inhibits HGF binding to the membrane, then the [^{125}I]-labeled HGF will not be in the proximity of the scintillation bead resulting in decreased signal. A secondary assay used below would determine if this compound is an agonist or antagonist.

To assess the effect of a test compound on cell acidification rates, mesangial cells are incubated in the presence or absence of HGF plus or minus the test compound. The acidification rates of the cells are measured by microphysiometry as described herein and the effect of the test compound on HGF-induced cell acidification is determined. A reduction in the acidification rates of cells treated with HGF and the test compound, compared with the acidification rates in cells treated with HGF alone, is an indication that the test compound has HGF antagonist activity.

To assess the effect of a test compound on extracellular matrix gene expression, cells are incubated in the presence or absence of HGF plus or minus the test compound under the conditions described herein in the experimental examples section. The expression of mRNA associated with extracellular matrix genes (e.g., collagen a1(IV) and fibronectin) is measured and compared among the different sets of cells. A reduction in extracellular matrix mRNA expression in cells treated with HGF and the test compound, compared with extracellular matrix mRNA expression in cells treated with HGF alone, is another indication that the test compound possesses HGF antagonist activity.

The test compound may also be tested for HGF antagonist activity in an *in vivo* assay. As will be apparent from the data provided in the experimental

examples section, long term treatment of mice with HGF induces renal failure. Thus, another exemplary way to assess the HGF antagonist activity of a test compound is to administer HGF alone HGF plus test compound to mice over at least a 21 day period. Additional controls include mice which are administered a suitable placebo compound. Creatine clearance is assessed in each set of mice as a measure of renal function. An increase in creatine clearance in mice administered HGF plus the test compound, compared with creatine clearance in mice administered HGF alone, is another indication that the test compound has HGF antagonist activity.

HGF antagonists which comprise peptides comprising specific portions of HGF include, but are not limited to, those which encompass the first krinkle domain near the N-terminal of HGF. An example of such a peptide is the truncated HGF peptide NK2 (Chan et al., 1991, Science 254:1382-1385).

To obtain a substantially pure preparation of a peptide comprising a portion of HGF, the peptide may be produced by cloning and expressing HGF DNA encoding the desired portion of HGF. HGF DNA and amino acid sequences are provided in Miyazawa et al., 1991, Eur. J. Biochem. 197:15-22 (SEQ ID NOs: 1 and 2). An isolated DNA encoding the desired portion of HGF is cloned into an expression vector and the protein is expressed therefrom. Procedures for cloning and expression of peptides are well known in the art and are described, for example, in Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, New York). Peptide so expressed may be obtained using ordinary peptide purification procedures well known in the art.

As used herein, the term "substantially pure" describes a compound, *e.g.*, a protein or polypeptide

which has been separated from components which naturally accompany it. Typically, a compound is substantially pure when at least 10%, more preferably at least 20%, more preferably at least 50%, more preferably at least 60%, more preferably at least 75%, more preferably at least 90%, and most preferably at least 98% of the total material (by volume, by wet or dry weight, or by mole percent or mole fraction) in a sample is the compound of interest. Purity can be measured by any appropriate method, *e.g.*, in the case of polypeptides by column chromatography, gel electrophoresis or HPLC analysis. A compound, *e.g.*, a protein, is also substantially purified when it is essentially free of naturally associated components or when it is separated from the native contaminants which accompany it in its natural state.

The present invention also includes analogs of peptides obtained according to the methods of the invention. Analogs can differ from naturally occurring peptides by conservative amino acid sequence differences or by modifications which do not affect sequence, or by both.

For example, conservative amino acid changes may be made, which although they alter the primary sequence of the peptide, do not normally alter its function. Conservative amino acid substitutions typically include substitutions within the following groups:

glycine, alanine;
valine, isoleucine, leucine;
aspartic acid, glutamic acid;
asparagine, glutamine;
serine, threonine;
lysine, arginine;
phenylalanine, tyrosine.

Modifications (which do not normally alter primary sequence) include *in vivo*, or *in vitro* chemical derivatization of peptides, *e.g.*, acetylation, or carboxylation. Also included are modifications of glycosylation, *e.g.*, those made by modifying the glycosylation patterns of a peptide during its synthesis and processing or in further processing steps; *e.g.*, by exposing the peptide to enzymes which affect glycosylation, *e.g.*, mammalian glycosylating or deglycosylating enzymes. Also embraced are sequences which have phosphorylated amino acid residues, *e.g.*, phosphotyrosine, phosphoserine, or phosphothreonine.

Also included are peptides which have been modified using ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimize solubility properties. Analogs of such peptides include those containing residues other than naturally occurring L-amino acids, *e.g.*, D-amino acids or non-naturally occurring synthetic amino acids. The peptides of the invention are not limited to products of any of the specific exemplary processes listed herein.

Thus, the present invention also relates to active fragments of HGF having HGF antagonistic activity. A specific polypeptide is considered to have HGF antagonistic activity if it inhibits the action of HGF as described herein.

As used herein, the term "fragment," as applied to a HGF peptide, will ordinarily be at least about 10 contiguous amino acids, typically at least about 20 contiguous amino acids, more typically at least about 50 continuous amino acids and usually at least about 78 contiguous amino acids in length.

Nucleic acid sequence complementary to HGF may be generated using the sequence of HGF provided in Nakamura

et al. (*supra*). Administration of antisense oligonucleotides to mammals is now common in the art and may be accomplished by using any of the administration techniques described herein. Nucleic acid complementary to nucleotides 78-950 of HGF (SEQ ID NO: 1) or portions thereof may be obtained by cloning HGF DNA or portions thereof into an expression vector such that RNA is expressed therefrom in the antisense orientation (i.e., complementary) to HGF mRNA.

An "isolated DNA", as used herein, refers to a DNA sequence, segment, or fragment which has been purified from the sequences which flank it in a naturally occurring state, *e.g.*, a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, *e.g.*, the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to DNA which has been substantially purified from other components which naturally accompany the DNA, *e.g.*, RNA or DNA or proteins which naturally accompany it in the cell.

"Complementary" as used herein, refers to the subunit sequence complementarity between two nucleic acids, e.g., two DNA or RNA molecules. When a nucleotide position in both of the molecules is occupied by nucleotides normally capable of base pairing with each other, then the nucleic acids are considered to be complementary to each other at this position. Thus, two nucleic acids are complementary to each other when a substantial number (at least 50%) of corresponding positions in each of the molecules are occupied by nucleotides which normally base pair with each other (e.g., A:T and G:C nucleotide pairs).

The invention should also be construed to include DNAs which are substantially homologous to HGF DNA or portions thereof, which DNAs are useful for the

production of HGF complementary nucleic acid, or for the production of HGF peptides. Preferably, DNA which is substantially homologous is about 50% homologous, more preferably about 70% homologous, even more preferably about 80% homologous and most preferably about 90% homologous to DNA obtained using the method of the invention.

"Homologous" as used herein, refers to the subunit sequence similarity between two polymeric molecules, *e.g.*, between two nucleic acid molecules, *e.g.*, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then they are homologous at that position. The homology between two sequences is a direct function of the number of matching or homologous positions, *e.g.*, if half (*e.g.*, five positions in a polymer ten subunits in length) of the positions in two compound sequences are homologous then the two sequences are 50% homologous, if 90% of the positions, *e.g.*, 9 of 10, are matched or homologous, the two sequences share 90% homology. By way of example, the DNA sequences 3'ATTGCC5' and 3'TATGCG5' share 50% homology.

Anti-HGF antibodies are easily generated by immunization of a mammal with the HGF peptide identified herein. Protocols for the generation of antibodies (either monoclonal or polyclonal antibodies) to a known peptide are described in Harlow et al. (1988, In: Antibodies, A Laboratory Manual, Cold Spring Harbor, NY), which protocols can be easily followed by the skilled artisan. Polyclonal antibodies to HGF may be raised in any suitable mammal, such as a mouse or a rabbit. Monoclonal anti-HGF antibodies are generated by

immunization of a mouse with HGF peptide followed by production of hybridoma cells capable of secreting anti-HGF antibody. Other means of producing monoclonal antibodies, such as antibodies which are expressed by bacteriophage, are now also well known in the art and are described, for example, in Marks *et al.* (1991, *J. Mol. Biol.* 222:581-597), Barbas (1995, *Nature Medicine* 1:837-839), de Kruif *et al.* (1995, *J. Mol. Biol.* 248:97-105) and Sternberg *et al.* (1995, *Proc. Natl. Acad. Sci. USA* 92:1609-1613).

Peptidometics having HGF-antagonist-like activity may also be designed and used according to the present invention. Additional information describing administration of peptidometics is provided in PCT/US93/01201 and U.S. Patent No. 5,334,702, which are hereby incorporated herein by reference. Any of the techniques described in either of these two references may be employed in the present invention for the administration of peptidometics.

An HGF antagonist may also include small molecules having HGF antagonist activity as defined herein which are not peptide or nucleic acid molecules.

Examples of HGF antagonists useful in the method of the present invention include, but are not limited to, those described in Faletto et al. (WO 94/06909) Roos et al. (WO94/06456), JP05208998 and Aaronson et al., (WO 92/05184).

Kidney diseases which are treatable using the method of the invention include, but are not limited to, polycystic disease, diabetic nephropathy, focal segmental glomerulosclerosis, hypertension-induced nephropathy, hypernephroma, and the like.

Protocols for treatment of mammals with a chronic renal disease involving administration of an antagonist

of HGF will be apparent to those skilled in the art and will vary depending upon the type of disease and the type and age of the mammal. Treatment regimes which are contemplated include a single dose or dosage which is administered hourly, daily, weekly or monthly, or yearly. Dosages may vary from 1-1000 mg/kg of body weight of the antagonist, and will be in a form suitable for delivery of the compound. The route of administration may also vary depending upon the disorder to be treated.

The invention contemplates administration of an HGF antagonist to humans for the purpose of alleviating or ablating chronic renal disease. The protocol which is described below for administration of HGF to a human is provided as an example of how to administer HGF to a human. This protocol should not be construed as being the only protocol which can be used, but rather, should be construed merely as an example of the same. Other protocols will become apparent to those skilled in the art when in possession of the instant invention. Essentially, for administration to humans, the HGF antagonist is dissolved in about 1 ml of saline and doses of 1-1000 mg per kg of body weight are administered orally or intra-venously once per day to several times per day. Renal function is monitored throughout the administration period.

The antagonist of HGF is prepared for administration by being suspended or dissolved in a pharmaceutically acceptable carrier such as saline, salts solution or other formulations apparent to those skilled in such administration. The compositions of the invention may be administered to a mammal in one of the traditional modes (e.g., orally, parenterally, transdermally or transmucosally), in a sustained release formulation using a biodegradable biocompatible polymer, or by on-site delivery using micelles, gels and

liposomes, or rectally (e.g., by suppository or enema) or nasally (e.g., by nasal spray). Thus, an HGF antagonist may be administered to the mammal by any route in order that it eventually reaches the target area in the mammal, i.e., the kidney, wherein it exerts its effects. The appropriate pharmaceutically acceptable carrier will be evident to those skilled in the art and will depend upon the route of administration.

Compounds having HGF antagonist activity also include compounds which are formulated so as to target specific types of cells. For example, it is now known in the art to encapsulate or otherwise formulate compounds such that they are directed to specific receptors on cells. Such formulations include antibody-tagging formulations, receptor-ligand binding formulations and the like.

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

The experimental examples described herein provide procedures and results which establish that antagonists of HGF are useful for treatment of chronic renal disease since, according to the data provided herein, treatment of cells *in vitro* with HGF results in the synthesis in the production of compounds associated with chronic renal disease. Similarly, long term treatment of animals *in vivo* with HGF results in a reduction of renal function.

Examples

Cell culture

Mouse mesangial cell cultures were established from glomeruli obtained from the kidneys of 8 to 10 week old SJL/J ($H\text{-}2^B$) mice (Wolf et al., 1992, Am. J. Pathol. 140:95-107). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 2 mM L-glutamine and 180 mg/dl glucose, supplemented with 10% FBS and 100U/ml penicillin/streptomycin at 37°C in 5% CO_2. Cells were subcultured by rinsing with phosphate buffered saline (PBS) and incubation with 0.05% trypsin supplemented with 20 mM EDTA.

Mouse mesangial cells transformed with noncapsid forming SV40 virus to establish a permanent cell line (Wolf et al., *supra*) are designated as MMC-SV40 cells. These cells exhibit many features of differentiated mesangial cells (Wolf et al., *supra*). A stable transfection was performed on MMC-SV40 cells with a reporter construct HB35, which expresses luciferase driven by a "minigene" comprised of the 5' flanking and first intron regions of the murine COL4A1 gene (Fumo et al., 1994, Am. J. Physiol. 267:F632-F638). The stable transformants are designated as MMC-COL cells. These cells exhibited patterns of growth and protein synthesis in response to elevated glucose concentration similar to MMC-SV40 cells (Fumo et al., *supra*).

Cryopreserved human mesangial cells (passage 3) were purchased from Clonetics Corp. (San Diego, CA) and were grown in Clonetics Mesangial Cell Growth Medium (MsGM) supplemented with 5% FBS, 50 mg/ml Gentamicin and 50 ng/ml Amphotericin-B. Human mesangial cells were grown according to the methods provided by Clonetics Corp. and were used in these experiments between passage 6-8.

Microphysiometry

The cytosensor microphysiometer is based on a pH sensitive silicon sensor which is part of a microvolume flow chamber in which cells are immobilized (McConnel et al., 1992, Science 257:1906-1912). Mouse mesangial cells were subcultured with trypsin from 150 cm^2 flasks into capsule cups (Molecular Devices, Inc., Sunnyvale, CA) having a polycarbonate membrane of 3 mm pore size at a density of 300,000 cells per cup. Cells were allowed to attach for 24 hours in the medium specified for each cell type. After spacer rings and insert cups were fitted into the capsule cups, the assembled units were transferred to the sensor chamber and perfused at 100 ml/min with bicarbonate-free RPMI 1640 medium (Molecular Devices, Inc.) in the microphysiometer. Acidification rate was measured as a change in pH overtime, which was determined when the pumps were turned off for 30 seconds in 2 minute intervals.

Mitogenesis assay

The mitogenic effect of HGF on mouse mesangial cells was measured as the amount of [^{3}H] thymidine incorporated into newly synthesized DNA. Cells were subcultured into 24 well dishes (2.5 x 10^3 cells per well and Incubated in growth media for 72 hours. Subconfluent cultures were made quiescent by placing them for 48 hours in DMEM medium containing 2 mM L-glutamine and 100 mg/dl glucose, the medium being further supplemented with 3% FBS and 100/ml penicillin and 100 mg/ml streptomycin. HGF was diluted in unsupplemented DMEM medium and then added to wells in triplicate for 24 hours. Cells were pulsed with [^{3}H] thymidine during the last 4 hours of the 24 hour incubation period. Cells were washed with PBS, and then 5% trichloroacetic acid was added to precipitate

proteins and nucleic acids and to remove unincorporated [^{3}H] thymidine. The precipitate was then dissolved by adding 0.5 ml of 0.5 N NaOH and 400 ml aliquots were added to scintillation fluid and counted on a Taurus liquid scintillation counter (ICN Biomedicals, Inc., Huntsville, AL).

Luciferase assay

MMC-COL cells were cultured in 24 well plates at 25,000 cells per well in growth medium for 48 hours. Cells were then incubated in the same medium used to make the cells quiescent in the proliferation assay and were incubated for an additional 48 hours before HGF was added. At the times indicated, cells were lysed using 500 ml of a buffer containing 0.1 mM potassium phosphate and 1 mM dithiothreitol, pH 7.2 with 1 % Triton X-100 (luciferase activity) or were trypsinized (cell number). The lysed cells were centrifuged and 100 ml of the supernatant was added in duplicate to wells of a 96 well microliter plate. Light emission was measured directly at room temperature using a Microlumat LB96P luminometer (Wallac Inc., Gaithersburg, MD) and integrated over a 20-s period following the automated injection of 100 ml of a luciferin reaction mixture. This mixture contained a stock buffer of 0.1 mM potassium phosphate, 10 mM ATP, 20 mM $MgCl_2$ and 1 mM dithiothreitol, pH 7.2, and freshly added 0.8 mg/ml of D-luciferin (Boehringer Mannheim, Indianapolis, IN). Luciferase activity was expressed as relative light units (RLU) per number of cells as determined from similarly treated cultures of cells in wells which were trypsinized and counted (Coulter Electronic LTD, Luton, Beds, England).

Northern blot hybridization

MMC-SV40 cells were cultured in 150 cm^2 flasks and incubated for 3 to 4 days in the growth medium described above. The medium was changed to DMEM medium containing 2 mM L-glutamine and 100 mg/dl glucose and supplemented with 3% FBS and 100U/ml penicillin and 100 mg/ml streptomycin for 48 hours before the addition of HGF. Cells were incubated for 2, 6, and 16 hours with HGF. Total RNA was extracted from mouse cells by guanidinium thiocyanate denaturation and acidified phenol-chloroform extraction (Chomczynski et al., 1987, Anal. Biochem. 162:156-159). Total RNA (10 mg/lane) was fractionated on 0.2 M formaldehyde-1% agarose gels and transferred to nylon membranes (Nylon-1; Bethesda Research Laboratories, Bethesda, MD) in 4X SSC. Equivalent loading and transfer were verified by methylene blue staining. Random primed [^{32}P]DNA probes were made for fibronectin that recognize an mRNA of 7.6 kb. The fibronectin clone was purchased from ATCC. Hybridizations were performed with 10^6 cpm/ml of labeled DNA in 50% formamide, 225 mM NaCl, 20 mM NaH_2PO_4, 1.5 mM EDTA, 1% sodium dodecyl sulfate, 0.5% dry milk, 100 mg/ml yeast total RNA and 300 mg/ml salmon DNA, at 42°C for 16 hours. Blots were washed with a final stringency of 0.2 X SSC, 0.2% sodium dodecyl sulfate at 65°C. Membranes were exposed to phosphor imaging plate and bands were quantified with ImageQuant software (Molecular Dynamics, Inc.).

Animal experiments

Alzet mini-osmotic pumps (Alza, Palo Alto, CA) were filled with either HGF at 14.6 ng/ml or vehicle (350 mM NaCl, 10 mM phosphate pH 7.3, 625 mg/ml human serum albumin. Lean and obese mice of the C57BL/Ks strain with the recessive db mutation were purchased from the

Jackson laboratory (Bar Harbor, ME). Mice received the implants in the peritoneal cavity under Ketalar (60 mg/kg) and were killed 21 days later. Twenty-four hour urine samples were collected in metabolic cages on the day of sacrifice. At the time of sacrifice, blood was also collected. Urine and serum creatinine levels were determined by a Synchron Clinical System AS8 (Beckman; Columbia, MD). Renal function was determined by calculating creatinine clearance.

HGF mediated acidification rates

In order to evaluate the effect of FBS or BSA on HGF mediated acidification rates, MMC-SV40 cells were pretreated with 0.1% BSA, 0.5% FBS or both for 30 minutes. This experiment was performed because recombinant HGF obtained from R&D Systems, Minneapolis, MN occurs in the single chain precursor form which requires enzymatic activation. Proteases in the serum or proteases secreted by cells may activate HGF, or proteases contained in FBS may be required. In addition, HGF, like other growth factors, tends to adhere to plastic tubing and thus, a carrier molecule for delivery of HGF to the cells may be required. Following the pre-incubation period, cells were exposed to 100 mg/ml HGF for 10 minutes in the presence or absence of 0.1 % BSA or 0.5% FBS.

In the presence of FBS, a peak of HGF-induced acidification was observed to be attenuated although the baseline response was reset at 30% higher than prior to HGF administration. BSA caused a dramatic attenuation of the peak response to HGF with or without FBS (Figure 1, Panel A). Therefore, in the remaining experiments HGF was administered in the absence of FBS or BSA in the running medium.

The effect of the length of time of exposure of cells to HGF on acidification rates was assessed by treating MMC-SV40 cells with 100 ng/ml HGF for 2, 5, 10, and 15 minutes. While incubation of the cells in the presence of HGF for 5, 10, and 15 minutes each yielded similar peaks of acidification rates, a 5 minute exposure time was chosen for further experiments because of the rapid recovery of the cells following this treatment (Figure 1, Panel B).

The HGF dose response was assessed by treating MMC-SV40 cells for 5 minutes with 3, 10, 30, or 100 ng/ml of HGF. A concentration of 30 ng/ml HGF elicited the maximum increase in acidification rate with no further increase at 100 ng/ml HGF. (Figure 1, Panel C).

To determine if non-transformed mesangial cells also respond to HGF, primary human mesangial cells were treated at passage 7. HGF at a concentration of 100 ng/ml induced an increase in the acidification rate of human mesangial cells (Figure 1, Panel D).

The involvement of PKC mediated second messenger systems in mesangial responses to HGF was evaluated using the PKC inhibitor RO-32-0432. Transformed mouse mesangial cells were pre-treated with 1, 3, or 5 mM RO-32-0432 for 30 minutes and HGF at a concentration of 50 ng/ml was added for 5 minutes in the presence of the pretreatment medium. RO-32-0432 partially blocked the HGF-induced acidification rate in a concentration dependent fashion (Figure 2).

The effect of HGF on mitogenesis

Proliferation of normal human and immortalized mouse mesangial cells was evaluated by tritiated thymidine incorporation. Human glomerular mesangial cells were serum starved for 48 hours. Mouse mesangial cells were grown in 3% serum. Each set of cells was treated with increasing doses of HGF up to 100 ng/ml for

24 hours. HGF did not affect tritiated thymidine incorporation at any doses tested in both normal human and immortalized mouse mesangial cells (Figure 3).

The effect of HGF on extracellular matrix gene expression

The effect of HGF on extracellular matrix gene expression was evaluated by assessing collagen a1(IV) promoter activation of a luciferase reporter gene in MMC-COL cells and by northern blot hybridization analysis of collagen a1(IV) and fibronectin mRNA in MMC-SV40 cells.

HGF-induced activation of the collagen promoter in mouse mesangial cells in a dose-dependent fashion. Collagen promoter activity doubled in the presence of 30 ng/ml HGF compared with untreated cells (Figure 4).

MMC-SV40 cells were treated with 50 ng/ml HGF in the presence or absence of 1 mM 5-amino-2-(4-aminoanilino)benzenesulfonic acid (ICN Biomedicals, Inc., Costa Mesa, CA), a protein kinase C inhibitor. Northern blots indicated that collagen a1 (IV) mRNA levels were increased in the mouse mesangial cells by HGF and that the inhibitor blocked the HGF-induced increase (data not shown).

MMC-SV40 cells were also treated with 50 ng/ml HGF for 2, 6, and 16 hours in the presence of 3% serum. Northern blots indicated that HGF induced an increase in fibronectin mRNA levels at 6 and 16 hours following HGF administration (data not shown).

The effect of HGF on renal function

The effect of HGF on renal function was determined by evaluating creatinine clearance following chronic HGF administration for 21 days in normal and diabetic mice. Creatinine clearance in normal and diabetic vehicle implanted mice was 400 and 454 ml/minute/100g,

respectively. HGF caused a decrease in creatinine clearance in both groups to 283 and 287* ml/minute/100 g, respectively (*p < 0.05 vs vehicle group; n = 5-6 mice; Figure 5).

The data presented herein establish that glomerular mesangial cells respond to HGF exhibiting changes in acidification rate and extracellular matrix gene expression. The effect of HGF on mesangial cells is mediated in part by protein kinase C second messenger systems.

In contrast to the mitogenic activity of HGF on epithelial cells, HGF has no effect on proliferation of mouse or human mesangial cells. HGF has distinct effects on glomerular mesangial cells during chronic renal failure. Moreover, in addition to other proteins known to affect renal malfunction, the present data demonstrate that HGF also contributes to matrix production in mesangial cells and therefore chronic renal disease.

In summary, HGF stimulates the extracellular acidification rate of mesangial cells and increases gene expression of fibronectin and collagen a1(IV) in glomerular mesangial cells. Therefore, HGF contributes to glomerulosclerosis by activating mesangial cells to increase extracellular matrix deposition. Further, treatment of animals for 21 days with HGF (long term treatment) reduces creatinine clearance suggesting a reduction in renal function. Thus, chronic elevation of HGF observed in many chronic renal diseases likely contributes to decreased renal function by causing expansion of extracellular matrix in the glomerulus.

The disclosures of each and every patent, patent application and publication cited herein are hereby incorporated herein by reference in their entirety.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Brooks, David
Laping, Nicholas

(ii) TITLE OF THE INVENTION: HEPATOCYTE GROWTH FACTOR ANTAGONISTS

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SmithKline Beecham Corporation
(B) STREET: 709 Swedeland Road
(C) CITY: King of Prussia
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19406

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: HEREWITH
(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/030,342
(B) FILING DATE: 05-NOV-1996

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Baumeister, Kirk
(B) REGISTRATION NUMBER: 33,833
(C) REFERENCE/DOCKET NUMBER: P50585P

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 610-270-5096
(B) TELEFAX: 610-270-5090
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1201 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 78...947
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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                   Met Trp Val Thr Lys Leu Leu Pro Ala Leu Leu
                    1               5                  10

CTG CAG CAT GTC CTC CTG CAT CTC CTC CTG CTC CCC ATC GCC ATC CCC       158
Leu Gln His Val Leu Leu His Leu Leu Leu Leu Pro Ile Ala Ile Pro
            15                  20                  25

TAT GCA GAG GGA CAA AGG AAA AGA AGA AAT ACA ATT CAT GAA TTC AAA       206
Tyr Ala Glu Gly Gln Arg Lys Arg Arg Asn Thr Ile His Glu Phe Lys
        30                  35                  40

AAA TCA GCA AAG ACT ACC CTA ATC AAA ATA GAT CCA GCA CTG AAG ATA       254
Lys Ser Ala Lys Thr Thr Leu Ile Lys Ile Asp Pro Ala Leu Lys Ile
    45                  50                  55

AAA ACC AAA AAA GTG AAT ACT GCA GAC CAA TGT GCT AAT AGA TGT ACT       302
Lys Thr Lys Lys Val Asn Thr Ala Asp Gln Cys Ala Asn Arg Cys Thr
60                  65                  70                  75

AGG AAT AAA GGA CTT CCA TTC ACT TGC AAG GCT TTT GTT TTT GAT AAA       350
Arg Asn Lys Gly Leu Pro Phe Thr Cys Lys Ala Phe Val Phe Asp Lys
                80                  85                  90

GCA AGA AAA CAA TGC CTC TGG TTC CCC TTC AAT AGC ATG TCA AGT GGA       398
Ala Arg Lys Gln Cys Leu Trp Phe Pro Phe Asn Ser Met Ser Ser Gly
            95                  100                 105

GTG AAA AAA GAA TTT GGC CAT GAA TTT GAC CTC TAT GAA AAC AAA GAC       446
Val Lys Lys Glu Phe Gly His Glu Phe Asp Leu Tyr Glu Asn Lys Asp
        110                 115                 120

TAC ATT AGA AAC TGC ATC ATT GGT AAA GGA CGC AGC TAC AAG GGA ACA       494
Tyr Ile Arg Asn Cys Ile Ile Gly Lys Gly Arg Ser Tyr Lys Gly Thr
    125                 130                 135

GTA TCT ATC ACT AAG AGT GGC ATC AAA TGT CAG CCC TGG AGT TCC ATG       542
Val Ser Ile Thr Lys Ser Gly Ile Lys Cys Gln Pro Trp Ser Ser Met
140                 145                 150                 155

ATA CCA CAC GAA CAC AGC TTT TTG CCT TCG AGC TAT CGG GGT AAA GAC       590
Ile Pro His Glu His Ser Phe Leu Pro Ser Ser Tyr Arg Gly Lys Asp
                160                 165                 170

CTA CAG GAA AAC TAC TGT CGA AAT CCT CGA GGG GAA GAA GGG GGA CCC       638
Leu Gln Glu Asn Tyr Cys Arg Asn Pro Arg Gly Glu Glu Gly Gly Pro
            175                 180                 185

TGG TGT TTC ACA AGC AAT CCA GAG GTA CGC TAC GAA GTC TGT GAC ATT       686
Trp Cys Phe Thr Ser Asn Pro Glu Val Arg Tyr Glu Val Cys Asp Ile
        190                 195                 200

CCT CAG TGT TCA GAA GTT GAA TGC ATG ACC TGC AAT GGG GAG AGT TAT       734
Pro Gln Cys Ser Glu Val Glu Cys Met Thr Cys Asn Gly Glu Ser Tyr
    205                 210                 215

CGA GGT CTC ATG GAT CAT ACA GAA TCA GGC AAG ATT TGT CAG CGC TGG       782
Arg Gly Leu Met Asp His Thr Glu Ser Gly Lys Ile Cys Gln Arg Trp
220                 225                 230                 235
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Asp His Gln Thr Pro His Arg His Lys Phe Leu Pro Glu Arg Tyr Pro
                240                 245                 250

GAC AAG GGC TTT GAT GAT AAT TAT TGC CGC AAT CCC GAT GGC CAG CCG      878
Asp Lys Gly Phe Asp Asp Asn Tyr Cys Arg Asn Pro Asp Gly Gln Pro
            255                 260                 265

AGG CCA TGG TGC TAT ACT CTT GAC CCT CAC ACC CGC TGG GAG TAC TGT      926
Arg Pro Trp Cys Tyr Thr Leu Asp Pro His Thr Arg Trp Glu Tyr Cys
        270                 275                 280

GCA ATT AAA ACA TGC GAG ACA TAACATGGGC TCTCAACTGA TGGTGAACTT CTTCT    982
Ala Ile Lys Thr Cys Glu Thr
    285                 290

GGTGAGTGAC AGAGGCTGCA GTGAAGAATA ATGAGTCTAA TAGAAGTTTA TCACAGATGT   1042
CTCTAATCTC TATAGCTGAT CCCTACCTCT CTCGCTGTCT TTGTACCCAG CCTGCATTCT   1102
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 290 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Leu His Leu Leu Leu Leu Pro Ile Ala Ile Pro Tyr Ala Glu Gly Gln
            20                  25                  30
Arg Lys Arg Arg Asn Thr Ile His Glu Phe Lys Lys Ser Ala Lys Thr
        35                  40                  45
Thr Leu Ile Lys Ile Asp Pro Ala Leu Lys Ile Lys Thr Lys Lys Val
    50                  55                  60
Asn Thr Ala Asp Gln Cys Ala Asn Arg Cys Thr Arg Asn Lys Gly Leu
65                  70                  75                  80
Pro Phe Thr Cys Lys Ala Phe Val Phe Asp Lys Ala Arg Lys Gln Cys
                85                  90                  95
Leu Trp Phe Pro Phe Asn Ser Met Ser Ser Gly Val Lys Lys Glu Phe
            100                 105                 110
Gly His Glu Phe Asp Leu Tyr Glu Asn Lys Asp Tyr Ile Arg Asn Cys
        115                 120                 125
Ile Ile Gly Lys Gly Arg Ser Tyr Lys Gly Thr Val Ser Ile Thr Lys
    130                 135                 140
Ser Gly Ile Lys Cys Gln Pro Trp Ser Ser Met Ile Pro His Glu His
145                 150                 155                 160
Ser Phe Leu Pro Ser Ser Tyr Arg Gly Lys Asp Leu Gln Glu Asn Tyr
                165                 170                 175
Cys Arg Asn Pro Arg Gly Glu Glu Gly Gly Pro Trp Cys Phe Thr Ser
            180                 185                 190
Asn Pro Glu Val Arg Tyr Glu Val Cys Asp Ile Pro Gln Cys Ser Glu
        195                 200                 205
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Val Glu Cys Met Thr Cys Asn Gly Glu Ser Tyr Arg Gly Leu Met Asp
    210                 215                 220
His Thr Glu Ser Gly Lys Ile Cys Gln Arg Trp Asp His Gln Thr Pro
225                 230                 235                 240
His Arg His Lys Phe Leu Pro Glu Arg Tyr Pro Asp Lys Gly Phe Asp
                245                 250                 255
Asp Asn Tyr Cys Arg Asn Pro Asp Gly Gln Pro Arg Pro Trp Cys Tyr
            260                 265                 270
Thr Leu Asp Pro His Thr Arg Trp Glu Tyr Cys Ala Ile Lys Thr Cys
        275                 280                 285
Glu Thr
    290
```

Claims

1. A method of identifying an antagonist of HGF activity comprising:

treating cells with HGF in the presence or absence of a test compound; and

comparing the level of fibronectin mRNA in the cells, wherein a lower level of fibronectin mRNA in the cells in the presence of the test compound compared with the level of fibronectin mRNA in the absence of the test compound is an indication that the test compound is an HGF antagonist.

2. A method of identifying an antagonist of HGF activity comprising:

treating cells with HGF in the presence or absence of a test compound; and

comparing the acidification rate in the cells, wherein a lower acidification rate in the cells in the presence of the test compound compared with the acidification rate in the absence of the test compound is an indication that the test compound is an HGF antagonist.

3. A method of identifying an antagonist of HGF activity comprising:

administering HGF to mice in the presence or absence of a test compound; and

comparing creatine clearance rates in the mice, wherein an increase in the rate of creatine clearance in mice administered HGF and the test compound compared with the rate of creatine clearance in mice administered HGF alone is an indication that the test compound is an HGF antagonist.

4. An HGF antagonist identified by the method of claim 1, 2 or 3.

5. A method of treating a human with chronic renal disease comprising administering an HGF antagonist to the human.

6. An HGF antagonist selected from the group consisting of an anti-HGF antibody, a peptidometic and a polypeptide fragment of HGF.

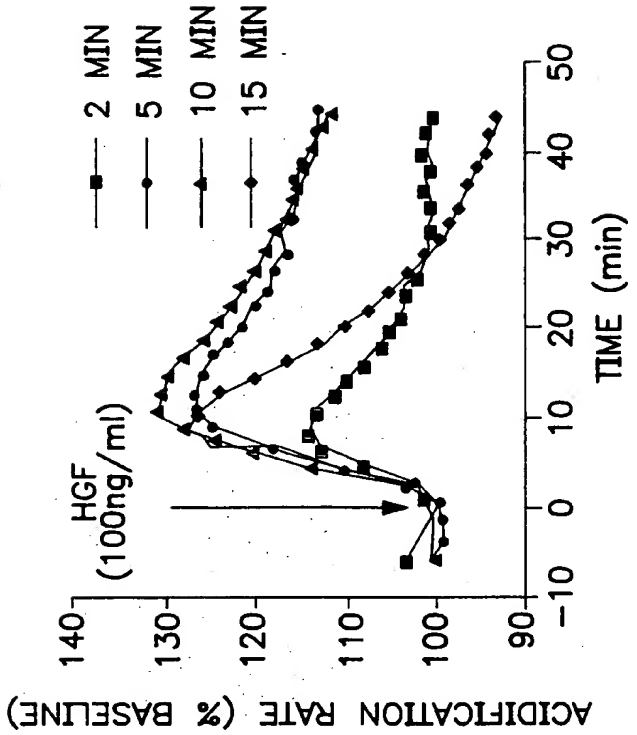

2 MIN
5 MIN
10 MIN
15 MIN
HGF (100ng/ml)
ACIDIFICATION RATE (% BASELINE)
TIME (min)
140
130
120
110
100
90
−10
0
10
20
30
40
50

FIG. IB

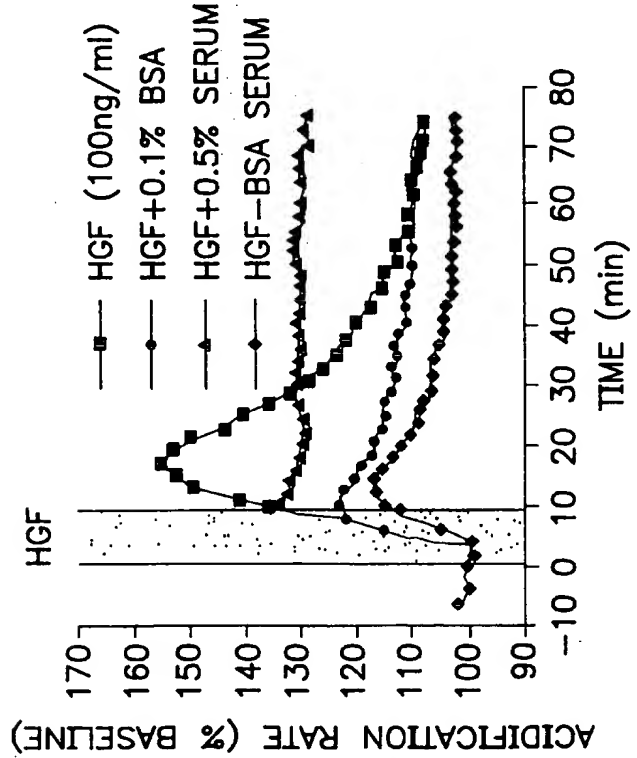

HGF (100ng/ml)
HGF+0.1% BSA
HGF+0.5% SERUM
HGF−BSA SERUM
HGF
ACIDIFICATION RATE (% BASELINE)
TIME (min)
170
160
150
140
130
120
110
100
90
−10
0
10
20
30
40
50
60
70
80

FIG. IA

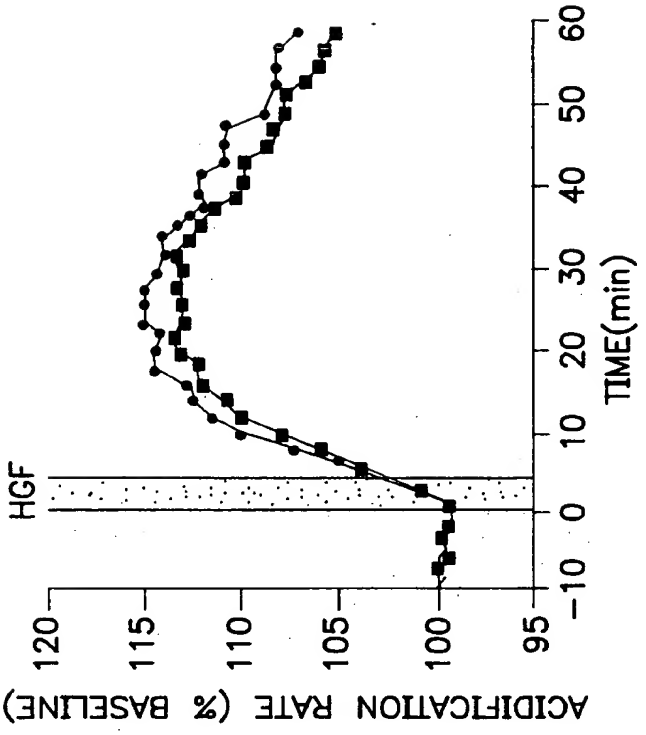
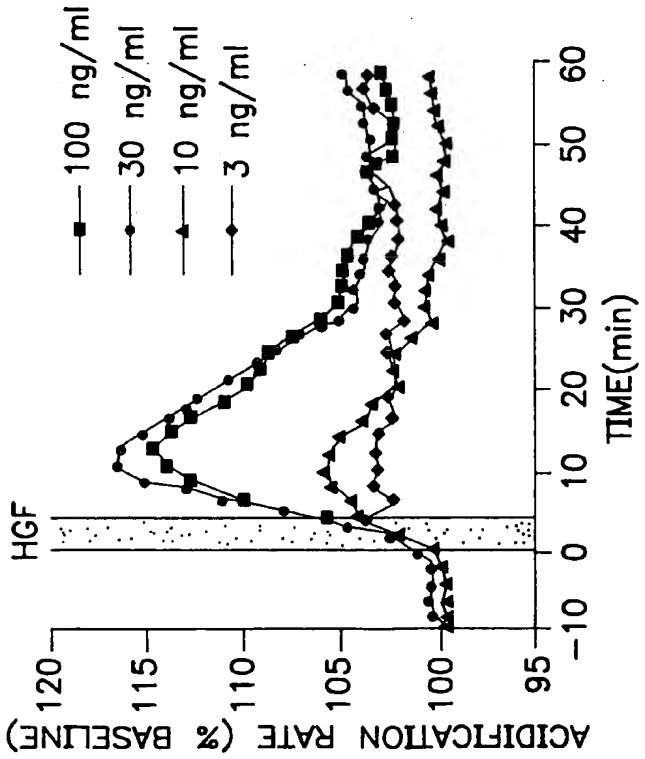

ACIDIFICATION RATE (% BASELINE)
120
115
110
105
100
95
HGF
−10
0
10
20
30
40
50
60
TIME(min)

FIG. ID


100 ng/ml
30 ng/ml
10 ng/ml
3 ng/ml
ACIDIFICATION RATE (% BASELINE)
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115
110
105
100
95
HGF
−10
0
10
20
30
40
50
60
TIME(min)

FIG. IC

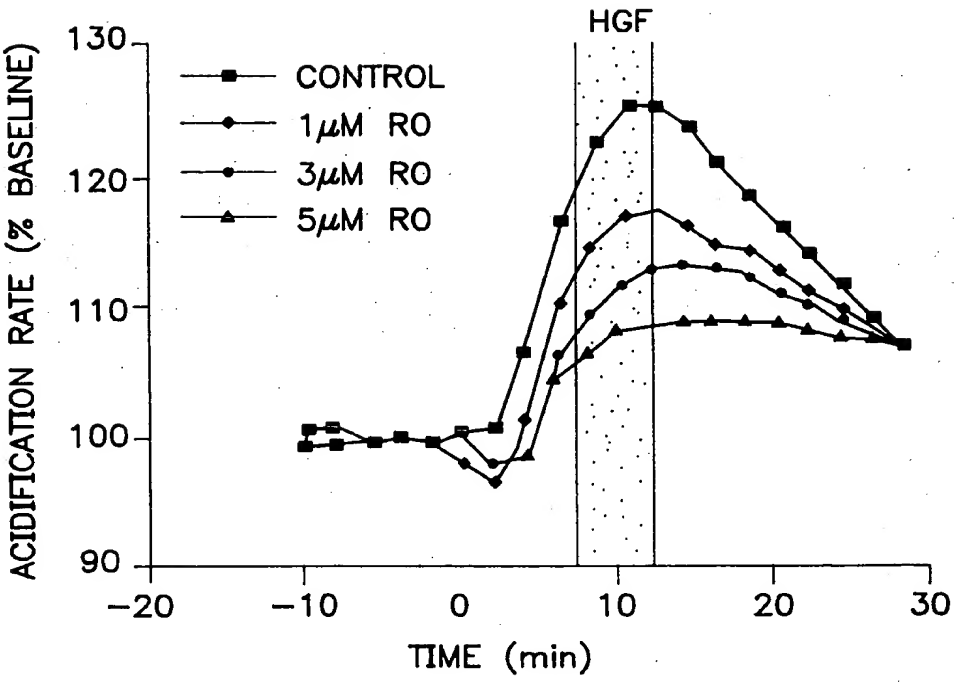
HGF
CONTROL
1μM RO
3μM RO
5μM RO
ACIDIFICATION RATE (% BASELINE)
130
120
110
100
90
−20
−10
0
10
20
30
TIME (min)

FIG. 2

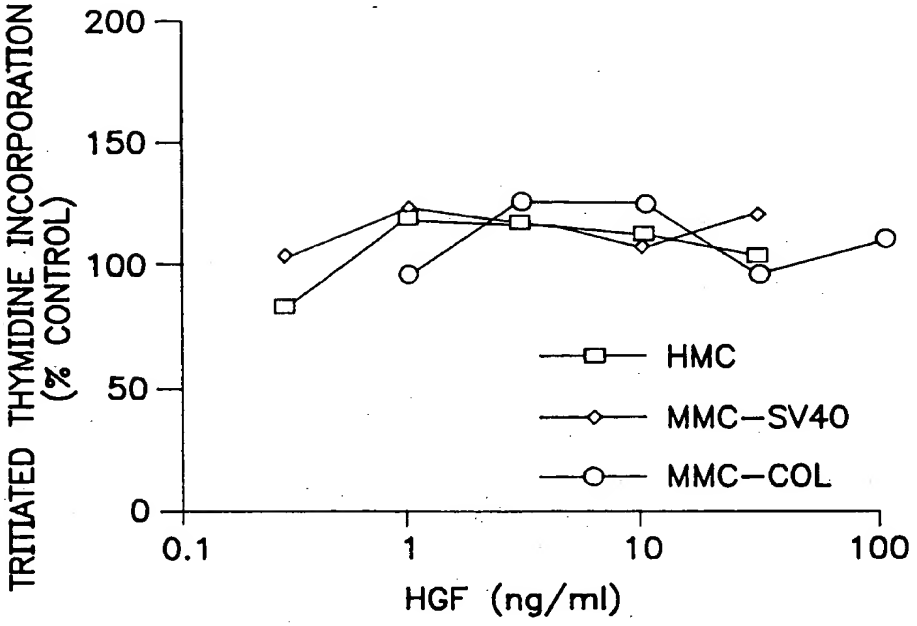
TRITIATED THYMIDINE INCORPORATION (% CONTROL)
200
150
100
50
0
0.1
1
10
100
HGF (ng/ml)
HMC
MMC–SV40
MMC–COL

FIG. 3

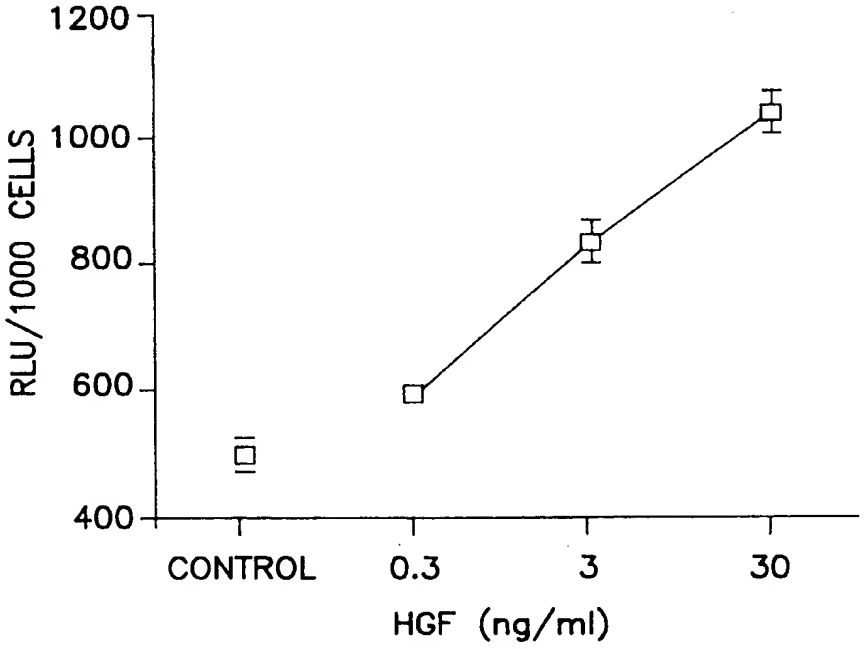
RLU/1000 CELLS
1200
1000
800
600
400
CONTROL
0.3
3
30
HGF (ng/ml)

FIG. 4

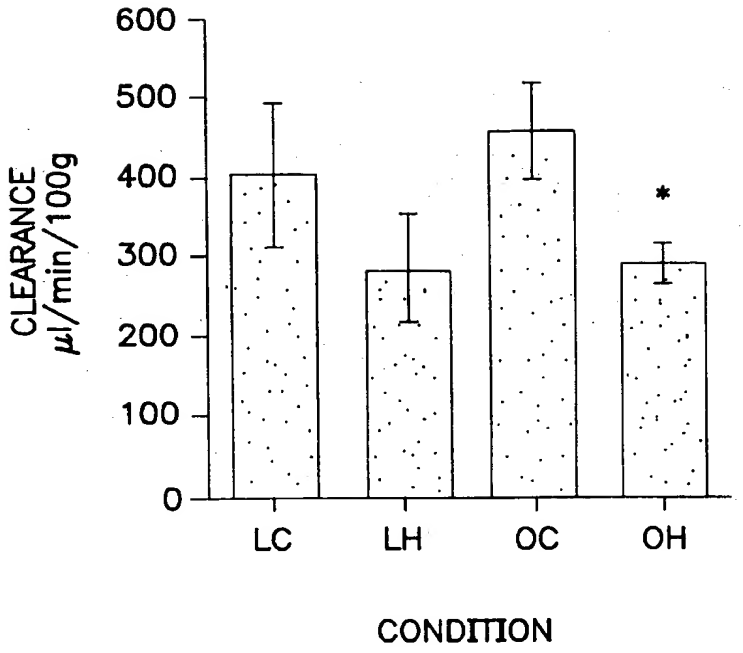
600
500
400
300
200
100
0
CLEARANCE
µl/min/100g
*
LC
LH
OC
OH
CONDITION

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/19891

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/18; C12Q 1/68; G01N 33/53
US CL :435/6, 7.1, 7.21; 514/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 7.21; 514/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS/USPAT; STN/Medline, HCaPlus
search terms: hepatocyte growth factor, hgf, scatter factor, antagonist#, fibronectin, creatine, acidif?, decreas? pH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GUILLEN, M.I. et al. The Hepatocyte Growth Factor Regulates the Synthesis of Acute-Phase Protein in Human Hepatocytes: Divergent Effect on Interleukin-6-Stimulated Genes. Hepatology. June 1996, Volume 23, pages 1345-1352, especially pages 1347 and 1349.	1
Y --- A	US 5,547,856 A (GODOWSKI et al.) 20 August 1996, column 23, line 60 to column 26, line 10.	1 --- 2-3
P, A	US 5,646,036 A (SCHWALL et al.) 08 July 1997, column 16, line 8 to column 17, line 41.	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 FEBRUARY 1998	Date of mailing of the international search report 09 MAR 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KAREN E. BROWN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/19891

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/19891

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-3, drawn to a method of identifying an antagonist of HGF.

Group II, claim(s) 4-6, drawn to an HGF antagonist and a method of treating a human with an HGF antagonist.

The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

PCT Rule 13.2 states that "An international or a national stage application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combinations of categories:

(1) A product and a process specially adapted for the manufacture of said product; or

(2) A product and a process of use of said product; or

(3) A product, a process specially adapted for the manufacture of the said product, and a use of the said product; or

(4) A process and an apparatus or means specifically designed for carrying out the said process; or

(5) A product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process."

The special technical feature of Group I is a method of identifying HGF antagonists. The process of Group I, although it identifies the antagonist of Group II, is not required for its manufacture. Furthermore, the process of Group II, which is drawn to a method of using an antagonist,does not share the same special technical feature as the method of Group I, because the process steps and reagents required for the method of Group I are different from that of Group II.

Therefore, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.